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Data in Brief

Genome-wide gene expression profiling of homeodomain-interacting protein kinase 2 deficient medullary thymic epithelial cells

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ABSTRACT

The establishment of central tolerance essentially depends on the promiscuous gene expression (pGE) of a plethora of tissue restricted antigens by the medullary thymic epithelial cells. The antigens are presented to developing thymocytes in the thymus to select for non-self reactive T-cell receptors in order to prevent autoimmune reactions in the periphery. However the molecular regulation of tissue-restricted antigen expression is still poorly understood. The only regulator known to play a role in the transcriptional regulation so far is the autoimmune regulator (AIRE). AIRE is thought to act in a multi-protein complex, promoting transcription, elongation and splicing of target genes. Yet the full composition of this Aire-associated multi-protein complex and its mode of action remain to be elucidated. Here we describe the experimental details and controls of the gene array analysis on the impact of the homeodomain-interacting protein kinase 2 (Hipk2) on promiscuous gene expression in medullary thymic epithelial cells based on the analysis of newly generated TEC-specific Hipk2 conditional knockout mice. The changes in gene expression are presumably mediated through a regulatory effect of Hipk2 on AIRE as published in the study by Rattay and colleagues in the Journal of Immunology [1]. The gene array data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE63432).

Specifications	
Organism/cell line/tissue	Mus musculus/medullary thymic epithelial cells
Sex	Young adult (4–6 weeks of age) females
Sequencer or array type	Illumina MouseWG-6 v2.0 Expression Bead Chip Sentrix arrays
Data format	Raw and analyzed
Experimental factors	TEC-specific Hipk2 conditional knock-out mice, Hipk2 deficient and mating wildtype control mice (floxed, Cre-), CD80 low and CD80 high mTECs
Experimental features	In duplicates, total RNA was obtained from isolated CD80 low and CD80 high mTECs each, of TEC-specific *Hipk2* knockout mice and mating wildtype mice as controls.

1. Direct link to deposited data

Deposited data can be found here: http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE63432.

2. Experimental design, materials and methods

2.1. Experimental design

Immature and mature medullary thymic epithelial cells (TECs) were isolated by FACS ($CD45^{-}EpCAM^{+}Ly51^{-}CD80^{low}$ or $CD80^{high}$ respectively) from TEC-specific conditional Hipk2 knockout mice (B6-Tg(Foxn1-cre)1Tbo Hipk2tm1Tgh) and internal mating control mice (floxed, Cre^{-}). The gene expression patterns in mTECs were compared between the Hipk2-deficient and control mice using Illumina bead arrays. The gene expression comparison between the knockout and the control mice of $CD80^{low}$ and $CD80^{high}$ mTECs was performed in two biological replicates each.

3. Material and methods

3.1. Mice

Young adult TEC-specific *Hipk2* conditional knockout (B6-Tg(Foxn1-cre)1Tbo Hipk2tm1Tgh) and control mice (floxed, Cre^{-}) of the age of 4–6 weeks were used. The *Hipk2* conditional knockout

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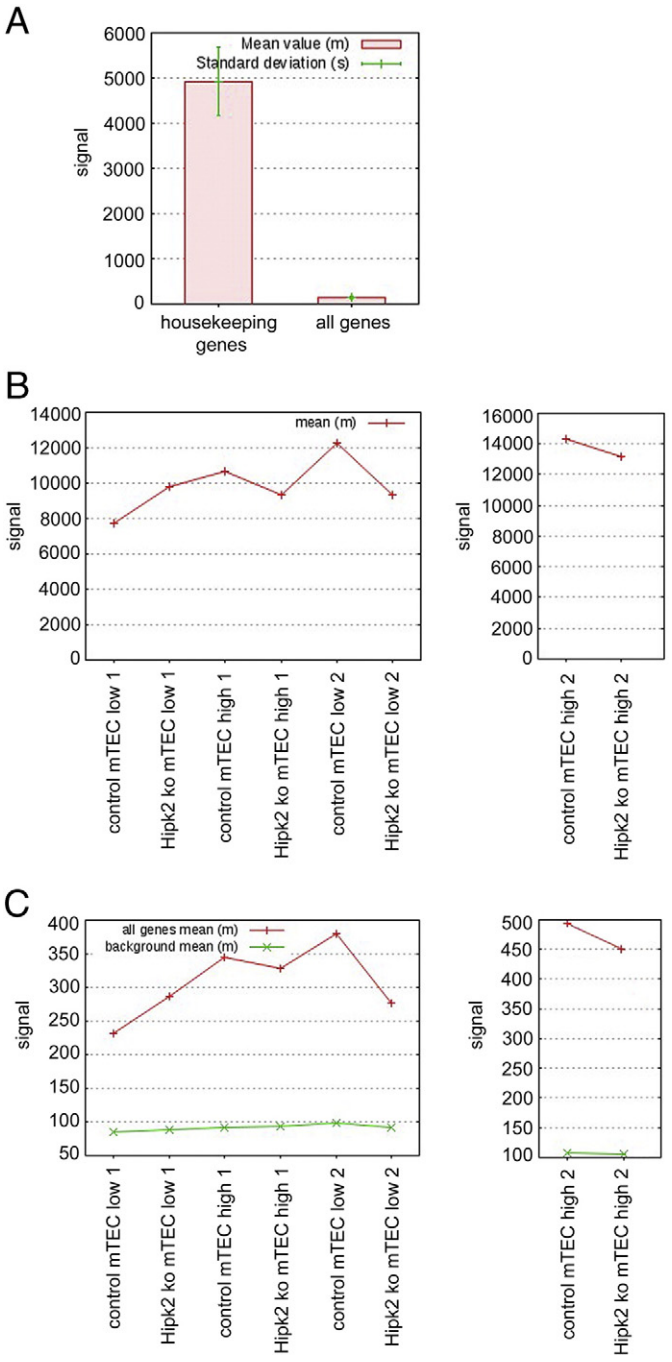

Fig. 1. Intensity controls comparing bead signals. (A) The signal intensity of the averaged housekeeping probes is shown in comparison to the signal intensity of all genes averaged. Shown is the mean with standard deviations, one of the two bead array chips is shown. (B) The signal intensity of the housekeeping gene probes is shown for each sample as mean values. (C) For each sample the signal intensity of all genes averaged is shown in comparison to the background signal levels. Shown are mean values.

mice (Hipk2$^{flox/flox}$; B6.CgHipk2tm1Tgh) were generated by Taconic Artemis GmbH (Köln, Germany). The detailed targeting strategy and genotyping protocol has been described [1].

3.2. Medullary thymic epithelial cell preparation

Thymi were collected, cleaned of fat and connective tissue and pooled from 10 to 15 mice for each experiment and cut into small pieces (approximately 1 mm diameter). After transfer into round-bottom tubes (Nunc) the thymic pieces were agitated under magnetic stirring in media (2% FCS, 1× Penicillin/Streptomycin, 10 mM Hepes in RPMI 1640 media) for 10 min at room temperature. The supernatant was discarded and the thymic pieces were digested both mechanically under magnetic stirring and enzymatically by two incubation steps in collagenase solution (0.2 mg/ml Collagenase type IV, 10 mM Hepes, 2% FCS in RPMI 1640 medium) for 15 min each at 37 °C followed by 3–5 digestion rounds in a collagenase/dispase enzyme mix (0.2 mg/ml Collagenase type IV, 0.2 mg/ml Dispase, 10 mM Hepes, 2% FCS, 25 μg/ml DNAse I in RPMI 1640 medium) for 25 min at 37 °C until the thymic pieces were completely dissolved. The collagenase/dispase fractions were pooled and filtered through a 40 μm nylon cell strainer.

In order to pre-enrich for CD45 negative thymic stromal cells the dissociated single cell suspension was depleted of CD45 positive cells by CD45 magnetic beads and autoMACS (Miltenyi Biotec).

Pre-enriched stromal cell fractions were stained with the following antibodies for isolation of TECs by FACS: anti-CD45-PE-Cy5 (clone 30-F11, BD), anti-EpCAM-A647 (G8.8 hybridoma [2]), anti-Ly51-FITC (clone 6C3, BD Pharmingen) and anti-CD80 (clone 16-10A1, BD Pharmingen). Dead cells were excluded by propidium iodide staining in a final concentration of 0.2 μg/ml. Cells were sorted on an Aria II cell sorter (BD). MTECs were defined as CD45$^-$Ly51$^-$EpCAM$^+$, of which the CD80^{low} and the CD80^{high} subsets were sorted separately.

3.3. RNA extraction and purification

Total RNA of primary sorted mTECs was isolated and purified using the High Pure RNA Isolation Kit (Roche Diagnostics) according to the manufacturers' protocol. The final elution step was performed using 50 μl of elution buffer.

The isolated RNA was precipitated by adding 1/10th volume of 3 M NaAcetat (in DEPC-H_2O; pH 5.2), 5 μg LPA and 2.5× sample volume of ice cold absolute ethanol for overnight incubation at −80 °C. After 16 hr, the sample was precipitated for further 2 hr at −20 °C to increase yield. Afterwards, the RNA was precipitated by centrifugation at 13,000 rpm for 5 min at 4 °C and the pellet was washed once by adding 500 μl icecold 70% EtOH (in DEPC-H_2O; pH 5.2). The supernatant was removed and the pellet was air-dried for 1–2 min at room temperature. The dry pellet was resuspended in 16 μl Nuclease-Free Water (Ambion) for gene array analysis.

3.4. RNA quality control

Prior to expression profiling on an Illumina bead gene array, RNA samples were tested for their quality by gel analysis using a total RNA Pico chip assay and Agilent 2100 Bioanalyzer (Agilent Technologies) to analyze their RIN (RNA integrity number) values. Depending on the amount and concentration of the RNA starting material either nano or pico chips were used for detection.

3.5. Microarray gene expression analysis

Whole genome expression analysis was performed using mouse WG-6 v2 BeadChip Sentrix arrays (Illumina). Labeling of the samples and hybridization were performed by the Genome and Proteome Core Facility of the DKFZ in Heidelberg.

Biotin-labeled cRNA for hybridization onto the mouse Sentrix-6 BeadChip array was prepared according to Illumina's recommended sample labeling procedure based on the modified Eberwine protocol [3]. In brief, 200 ng total RNA was used for cDNA synthesis, followed by an in vitro transcription step for amplification and labeling to synthesize biotin-labeled cRNA using the MessageAmp II RNA amplification kit (Ambion) and biotin-16-UTP (Roche). The cRNA was column purified using the TotalPrep RNA amplification kit (Illumina) and eluted in 45 μl water. The Quality of the cRNA was controlled by using a RNA Nano chip assay on an Agilent 2100 Bioanalyzer.

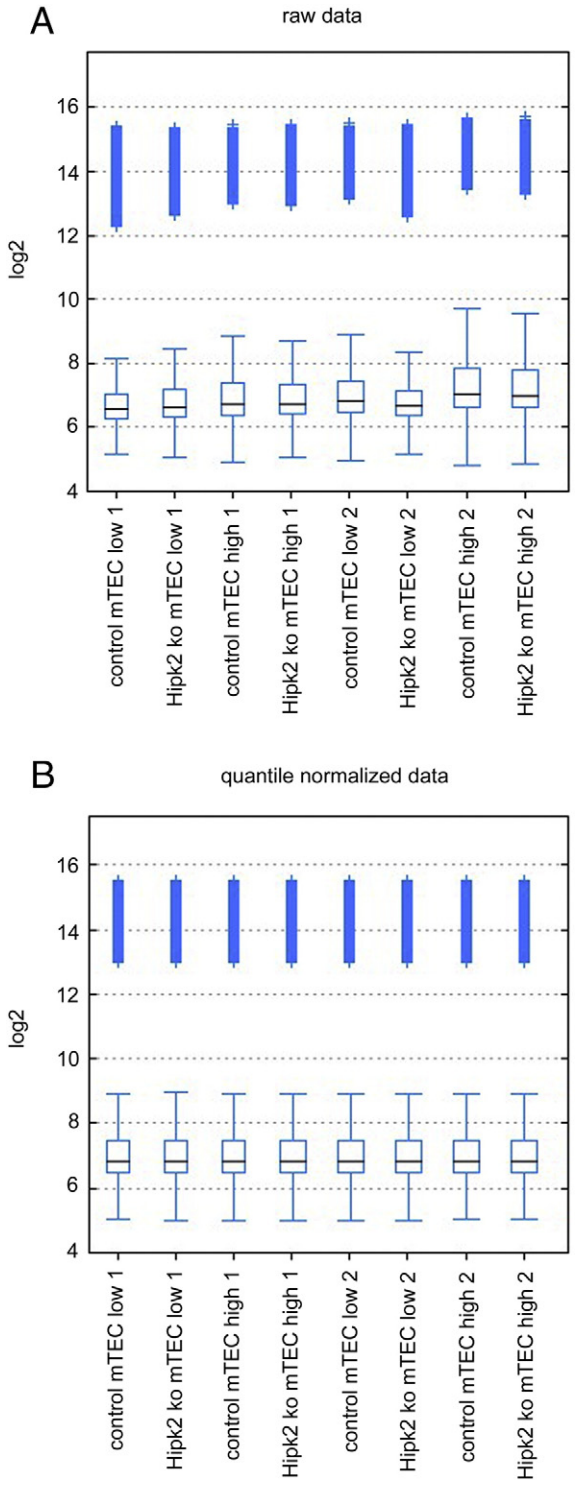

Fig. 2. Bead levels are compared between all samples. (A) Raw data bead levels of the arrays are shown. (B) Quantile normalized bead values of all samples are shown. Indicated are the quartiles (25–75%, lower boxes) and the maximum values (upper blue lines).

The hybridization to the chip was performed at 58 °C in GEX-HCB buffer (Illumina) at a concentration of 100 ng cRNA/μl, unsealed in a wet chamber for 20 hr. As controls, spike-in controls for low, medium and high abundant RNAs were added, as well as mismatch control and biotinylation contol oligonucleotides. The array chip was washed in High Temp Wash buffer (Illumina) at 55 °C for 10 min, followed by a wash in E1BC buffer (Illumina) at RT for 5 min, a wash in ethanol for 10 min and again a 2 min wash in E1BC buffer. Blocking was performed for 5 min in 4 ml of 1% (w/v) Blocker Casein in phosphate buffered saline Hammarsten grade (Pierce Biotechnology). Signal development was accomplished by a 10 min incubation in 2 ml of 1 μg/ml Cy3-streptavidin solution (Amersham Biosciences) and 1% blocking solution, followed by a final wash in E1BC prior drying and scanning the array chip.

Scanning of the microarray was done by using a Beadstation array scanner; settings were adjusted to a scaling factor of 1 and PMT settings at 430. The data extraction was done for all beads individually and outliers were removed when ≥2.5 MAD (median absolute deviation). All remaining data points were used for the calculation of the mean average signal for a given probe and the standard deviation for each probe was calculated. The gene expression intensity detected by the bead array was assessed for all samples (Fig. 1). The housekeeping signal intensity of all samples was above the averaged intensity of all genes within a sample. Further, the housekeeping and averaged all gene expression intensities were clearly separated from the background signal level.

3.6. Normalization and analysis details

The data were quantile normalized (Fig. 2) and differentially regulated genes were identified between HIPK2-deficient and control samples. The microarray analysis was performed in biological duplicates for $mTEC^{low}$ and $mTEC^{high}$ fractions on the $Hipk2^{ko}$ and control background. Genes with a fold change of ≥2 or ≤0.5 and with a p-value ≤0.0005 were considered to be differentially expressed. Statistical T-test calculation was performed in R, Benjamini-Hochberg correction is applied over all p-values of the differential expression analysis. The microarray data were deposited in GEO under accession number GSE63432.

4. Discussion

These data provide the first description of the effect of Hipk2 deficiency on promiscuous gene expression in mTECs. This dataset has been recently used in a study describing Hipk2 as an interaction partner of Aire, modulating its transcriptional activation function and thereby affecting gene expression in mTECs [1].

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